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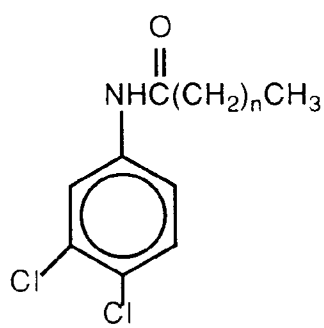
(54) **Antimicrobial compositions comprising 3,4-dichloroanilides and isothiazolones.**

(57) A synergistic antimicrobial composition comprising a 2-alkyl-3-isothiazolone and a 3,4-dichloroanilide of the formula



where n is an integer from 1 to 2, in a ratio to each other which exhibits synergism is disclosed.

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The present invention concerns antimicrobial compositions and methods of controlling microbes.

The presence of microbes in various aqueous systems such as latices, paints, coatings, cooling water systems, decorative ponds and the like, can cause deterioration or disfigurement of these systems. For example, painted surfaces may be disfigured by the unsightly buildup of microbes, thus detracting from the overall aesthetics of the painted article; cooling towers may lose efficiency due to the buildup of microbes such as algae on surfaces, thus reducing the heat transfer capabilities of the tower. It is conventional to practice methods which inhibit the microbial deterioration of such systems by incorporating a variety of additives or combination of additives that are characterized by having antimicrobial activity.

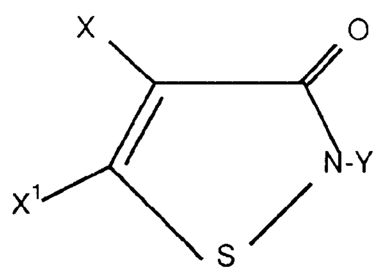
A wide variety of materials have been used to control microbes in different environments, some of which are: chlorine /bromine compounds, glutaraldehyde, isothiazolones, organotin formulations, copper salts, quaternary ammonium compounds (SD Strauss and PR Puckorius in *J. Power*, S1, june 1984), and triazines. Each has deficiencies related to toxicity, pH and temperature sensitivity, limited effectiveness, chemical stability, and/or compatibility.

Substituted anilides, e.g., 3,4-dichloropropionanilides, are known herbicides, but are not known for their antimicrobial properties.

Based on the aforementioned performance deficiencies of conventional antimicrobial compounds there is a need for more effective antimicrobial agents that can be used at lower dosage rates, thus being more cost effective for the end user, reducing the pollution load on the affected environmental systems, and reducing the side effects to nearby non-target organisms, such as fish, useful crops, etc.

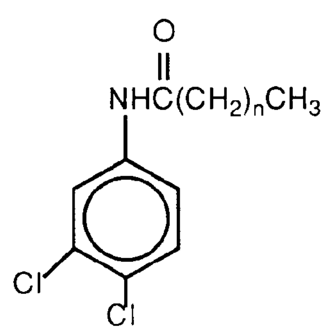
It is an object of this invention to provide a method of controlling microbes at very low levels of active ingredient. It is a further object to use compositions which are compatible with a variety of systems susceptible to deterioration by microbes. Another object is to provide a method of controlling microbes in cooling towers, paints, marine antifoulant coatings, spray washes, swimming pools, coatings, decorative ponds, fabric, leather, paper, wood, metal working fluids, cosmetic formulations, fuel systems, therapeutic pharmaceutical formulations and the like, without objectionable by-product odors, discoloration, or otherwise detrimental effects on the treated (and controlled) systems.

These objects, and others which will become apparent from the following disclosure, are achieved by the present invention which in one aspect provides a composition comprising
(A) a 2-alkyl-3-isothiazolone of the formula



(I)

wherein Y is $(C_5\text{-}C_{12})$alkyl or $(C_5\text{-}C_8)$cycloalkyl, and X and X^1 are each independently hydrogen, halogen, or $(C_1\text{-}C_4)$alkyl;
and (B) a 3,4-dichloroanilide of the formula



(II)

where n=1 or 2,
wherein the ratio of (A) to (B) is from 1/10000 to 100/1, preferably from 1/3300 to 1/10.

In another aspect, the invention provides a method for preventing or inhibiting the growth of bacteria, fungi, or algae in a locus susceptible or subject to contamination thereby, which comprises incorporating onto or into the locus, in an amount effective to adversely affect said growth, a composition as defined above.

The invention also encompasses coating or impregnant compositions and marine antifoulant compositions containing the above composition, and further includes the use of the above composition as a microbicide.

The specified ratios of (A) to (B) are selected because they produce an unexpected synergistic microbicidal effect between the two compounds.

The preferred isothiazolones are 4,5-dichloro-2-n-octyl-3-isothiazolone (Y = n-octyl, X and X^1 = chlorine) and 2-n-octyl-3-isothiazolone (Y = n-octyl, X and X^1 = hydrogen).

In contrast to the 3,4-dichloroanilides of formula II where n=1 or 2 (3,4-dichloropropionanilide and 3,4-dichlorobutyranilide, respectively), those anilides where n = 4 or greater do not provide combinations with the isothiazolones of formula I which are synergistic; in fact, several of these particular combinations are antagonistic towards one another in terms of antimicrobial activity (see Examples 3-6).

Another important utility is in imparting microbial resistance to a coating or impregnant composition by incorporation of the composition of the invention in the coating or impregnant, preferably at a concentration of about 0.1 ppm to about 2 percent, more preferably at concentration of about 1 ppm to 1 percent, and most preferably at a concentration of about 10 to 4000 ppm.

Algae-resistant coating or impregnant compositions provided by the invention preferably comprise about 0.1 ppm to about 2 percent of the antimicrobial composition, more preferably about 10 to 4000 ppm.

In a marine antifoulant composition, on the other hand, the antimicrobial composition of the invention comprises about 1 to 10 percent of the antifoulant composition.

In a therapeutic pharmaceutical formulation, e.g., lotion, cream, ointment or topical treatment; in the treatment of metal working fluids; and in the protection of fabric, leather, paper or wood materials, the microbicidal composition is generally added at a concentration of from about 0.1 ppm to about 2 percent by weight. In aqueous media, the microbial composition comprises from about 0.1 ppm to about 1 percent of the aqueous system depending on the specific end use; for examples, in cooling water tower applications and with pulp or paper manufacturing processes, the microbicidal composition is added at a concentration from about 0.1 to about 1000 ppm by weight. In cosmetic formulations, e.g., face or hand creams, toiletries, etc.; and int he treatment of fuel systems, e.g., jet fuel, gasoline, heating oil, etc., the microbicidal composition is added at a concentration of from about 0.1 ppm to about 1 percent by weight.

The microbial resistant compositions can also be used in construction products such as stucco, roof mastics, wall mastics, and masonry coatings for algae protection; in clear finishes and coatings to protect underlying substrates from algae; for algae control in aquaculture, including aquaria, fish hatcheries, shrimp ponds, finfish ponds, mollusc and crustacean cultivation; for algae control in recreational and decorative bodies of water such as swimming pools, lakes, fountains and decorative ponds; for algae control in bodies of water for industrial or municipal use, such as settling or separation ponds, waste treatment ponds, and water reservoirs; for algae control in hydroponic farming; for algae control in processing and manufacture of pulp and paper products; for inclusion in plastics or in coatings for plastics to protect against algae; and in plastics or coatings for plastics for swimming pool liners.

We prefer antimicrobial compositions wherein the weight ratio of (A) to (B) is from about 1/10000 to about 100/1. A preferred ratio range is from about 1/3300 to about 1/10 by weight. Particularly preferred ratio ranges are from about 1/200 to about 1/25 and from about 1/1000 to about 1/10 depending upon the particular isothiazolone/3,4-dichloroanilide combination in use.

The following examples represent just a few of the many uses and compounds of the invention. Various modifications, alternatives, and improvements should become apparent to those skilled in the art.

EXAMPLES

A. General Procedure

MIC values represent the Minimum Inhibitory Concentration. This is defined as the lowest level of compound required to completely inhibit (repress) the growth of a given organism.

A synergistic effect is defined as the response of two variables which is greater than the sum of both parts alone. Synergy was determined from combination studies with two compounds by the method of calculation described by F. C. Kull, P. C. Eisman, H. D. Sylwestrowicz and R. K. Mayer, *Applied Microbiology* 9,538 (1961):

$$\frac{Q_A}{Q_a} + \frac{Q_B}{Q_b} = \text{synergism index (SI)}$$

where:
Q_a = quantity of compound A, acting alone, producing an end point (MIC)
Q_A = quantity of compound A, in mixture, producing an end point (MIC)
Q_b = quantity of compound B, acting alone, producing an end point (MIC)
Q_B = quantity of compound B, in mixture, producing an end point (MIC)
The following SI values may be attained:

SI > 1 represents antagonistic effect,
SI = 1 represents additive effect,
SI < 1 represents synergy.

Efficacy studies were conducted on a variety of microorganisms with fatty acids and isothiazolone mixtures. The MIC studies were conducted using microtiter plate assays. In this method, a wide range of concentrations was tested by preparing two-fold serial dilutions of the compound in 96-well plastic microtiter plates. All liquid media transfers were performed with calibrated single or multichannel digital pipetters. Stock solutions of compounds were prepared in appropriate solvents and dispensed to the growth medium. All subsequent dilutions in plates were made using the desired growth medium; total volume of liquid in each well was 100 µl. Each plate contained a concentration of both compounds made by serially titrating equal volumes of liquids in two directions in the microtiter plate. Each plate contained a control row for each combination (one component only), hence, the individual compound MIC values were also determined.

B. Isothiazolone Structure

Isothiazolones included in the examples are designated as follows:
1) Isothiazolone A: 4,5-dichloro-2-n-octyl-3-isothiazolone
2) Isothiazolone B: 2-n-octyl-3-isothiazolone

C. Anilide Structure

3,4-Dichloroanilides evaluated for microbicidal activity in combination with Isothiazolones A and B included those according to formula II where
n=1 (3,4-dichloropropionanilide, Anilide A)
n=2 (Anilide B, 3,4-dichlorobutyranilide)
n=4 (Anilide C)
n=5 (Anilide D)
n=6 (Anilide E)
n=8 (Anilide F)

EXAMPLE 1

Using a pure culture of *Aspergillus niger*, various combinations of Anilide A (Compound B) and Isothiazolone A (Compound A) were subjected to MIC determinations in mycophil broth.

Qa	Qb	Qa/Qb	SI
0	125 (Q_B)	0	1.0
0.31	64	1/206	0.75
0.62	32	2/98	0.75
0.62	16	4/96	0.63
0.62	8	7/93	0.56
0.62	4	13/87	0.53
1.25 (Q_A)	0	-	1.0

EXAMPLE 2

Using a pure culture of *Aspergillus niger*, various combinations of Anilide B (Compound B) and Isothiazolone A (Compound A) were subjected to MIC determinations in mycophil broth.

Qa	Qb	Qa/Qb	SI
0	250 (Q_B)	-	1.0
0.62	125	1/195	0.75
1.25	62	2/98	0.75
1.25	31	4/96	0.63
2.5 (Q_A)	0	-	1.0

EXAMPLE 3 (Comparative)

Using a pure culture of *Aspergillus niger*, various combinations of Anilide C (Compound B) and Isothiazolone A (Compound A) were subjected to MIC determinations in mycophil broth.

Qa	Qb	Qa/Qb	SI
0	>1000 (Q_B)	-	1.0
10	500	2/98	>8
5	500	1/99	>4
10	250	4/96	>8
5	250	2/98	>4
10	125	7/93	>8
5	125	4/96	>4
2.5	125	2/98	>2
10	64	14/86	>8
5	64	7/93	>4
2.5	64	4/96	>2
1.25	64	2/98	>1
1.25 (Q_A)	0	-	1.0

EXAMPLE 4 (Comparative)

Using a pure culture of *Aspergillus niger*, various combinations of Anilide D (Compound B) and Isothiazolone A (Compound A) were subjected to MIC determinations in mycophil broth.

Qa	Qb	Qa/Qb	SI
0	>1000 (Q_B)	-	1.0
5	250	2/98	>2
5	125	4/96	>2
2.5	125	2/98	>1
5	64	7/93	>2
2.5	64	4/96	>1
5	32	14/86	>2
2.5	32	7/93	>1
2.5 (Q_A)	0	-	1.0

EXAMPLE 5 (Comparative)

Using a pure culture of *Aspergillus niger*, various combinations of Anilide E (Compound B) and Isothiazolone A (Compound A) were subjected to MIC determinations in mycophil broth.

Qa	Qb	Qa/Qb	SI
0	>1000 (Q_B)	-	1.0
10	500	2/98	>4
10	250	4/96	>4
5	250	2/98	>2
10	125	7/93	>4
5	125	4/96	>2
2.5	125	2/98	>1
10	64	14/86	>4
5	64	7/93	>2
2.5	64	4/96	>1
2.5 (Q_A)	0	-	1.0

EXAMPLE 6 (Comparative)

Using a pure culture of *Aspergillus niger*, various combinations of Anilide F (Compound B) and Isothiazolone A (Compound A) were subjected to MIC determinations in mycophil broth.

Qa	Qb	Qa/Qb	SI
0	>1000 (Q_B)	-	1.0
10	250	4/96	>4
10	125	7/93	>4
5	125	4/96	>2
10	64	14/86	>4
5	64	7/93	>2
10	32	24/76	>4
5	32	14/86	>2
2.5	32	7/93	>1
2.5 (Q_A)	0	-	1.0

EXAMPLE 7

Using a pure culture of *Aureobasidium pullalans*, various combinations of Anilide A (Compound B) and Isothiazolone A (Compound A) were subjected to MIC determinations in mycophil broth, pH = 5.0.

Qa	Qb	Qa/Qb	SI
0	250 (Q_B)	-	1.0
0.16	125	1/3120	0.63
0.32	125	1/1560	0.75
1.25 (Q_A)	0	-	1.0

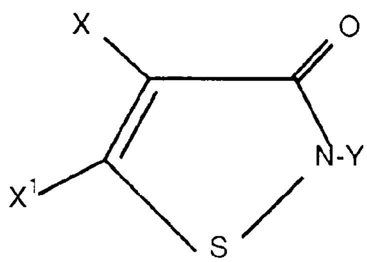
EXAMPLE 8

Using a pure culture of *Aureobasidium pullalans*, various combinations of Anilide A (Compound B) and Isothiazolone B (Compound A) were subjected to MIC determinations in mycophil broth, pH = 5.0.

Qa	Qb	Qa/Qb	SI
0	250 (Q_B)	-	1.0
0.16	125	1/800	0.56
0.32	125	1/400	0.63
0.64	125	1/200	0.75
1.25	64	2/98	0.75
1.25	32	4/96	0.63
1.25	16	7/93	0.56
1.25	8	14/86	0.53
2.5 (Q_A)	0	-	1.0

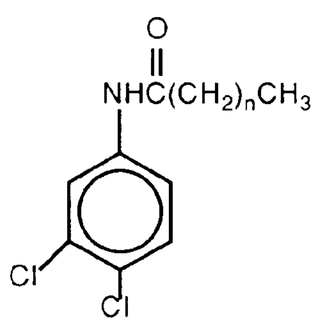
Claims

1. A composition comprising (A) a 2-alkyl-3-isothiazolone of the formula



(I)

wherein Y is $(C_5\text{-}C_{12})$alkyl or $(C_5\text{-}C_8)$cycloalkyl, and X and X^1 are each independently hydrogen, halogen, or $(C_1\text{-}C_4)$alkyl;
and (B) a 3,4-dichloroanilide of the formula



(II)

where n=1 or 2, wherein the ratio of (A) to (B) is from 1/10000 to 100/1, preferably from 1/3300 to 1/10.

2. Composition according to claim 1 wherein (A) is 4,5-dichloro-2-n-octyl-3-isothiazolone and (B) is 3,4-dichloropropionanilide, and the ratio of (A) to (B) is from 1/3300 to 1/10.

3. Composition according to claim 1 wherein (A) is 4,5-dichloro-2-n-octyl-3-isothiazolone and (B) is 3,4-dichlorobutyranilide, and the ratio of (A) to (B) is from 1/200 to 1/25.

4. Composition according to claim 1 wherein (A) is 2-n-octyl-3-isothiazolone and (B) is 3,4-dichloropropionanilide, and the ratio of (A) to (B) is from 1/100 to 1/10.

5. A coating or impregnant composition or therapeutic pharmaceutical composition comprising from 0.1 ppm

to 2 percent by weight, or a marine antifoulant composition comprising from 1 to 10 percent by weight, of a composition according to any preceding claim.

6. A method for preventing or inhibiting the growth of bacteria, fungi, or algae in a locus susceptible or subject to contamination thereby, which comprises incorporating onto or into the locus, in an amount effective to adversely affect said growth, a composition according to any preceding claim.

7. Method according to claim 6 wherein the locus is an aqueous medium, fuel system or cosmetic formulation and said composition is used in an amount of from 0.1 ppm to 1 percent by weight, or a coating or impregnant composition in which said composition is used in an amount of from 0.1 ppm to 2 percent by weight, or a marine antifoulant and said composition is used in an amount of from 1 to 10 percent by weight, or a pulp or paper manufacturing process and said composition is used in an amount of from 0.1 ppm to 1000 ppm by weight, or cooling tower water and said composition is used in an amount of from 0.1 ppm to 1000 ppm by weight, or a metal working fluid, topical therapeutic pharmaceutical formulation or fabric, leather, paper or wood and said composition is used in an amount of from 0.1 ppm to 2 percent by weight.

8. Use of a composition according to any of claims 1 to 5 as a microbicide.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 92301973.1

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	PATENT ABSTRACTS OF JAPAN, unexamined applications, section C, vol. 14, no. 356, August 2, 1990, THE PATENT OFFICE JAPANESE GOVERNMENT page 11 C 745 * Kokai-no. 02-129 105 (HOKKO CHEM IND CO LTD) * --	1,8	A 01 N 43/80 A 01 N 37/22
A	EP - A - 0 236 119 (SUTTON LABORATORIES, INC.) * Abstract * --	1,6,8	
A	US - A - 4 265 899 (LEWIS et al.) * Abstract * --	1,6,8	
A	GB - A - 2 225 944 (OSAMU UMEKAWA) * Abstract * ----	1,6,8	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

A 01 N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	09-06-1992	SCHNASS

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
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P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
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